CLAIMS

I claim:

1.   A method for producing a nucleic acid polymer suitable for expression of an amino acid sequence of interest, comprising:

(a)   cleaving two or more expression vectors to produce either non-palindromic ends or palindromic ends, wherein cleaved expression vectors with palindromic ends are further treated to produce non-palindromic ends, wherein the expression vectors comprise an expression cassette that comprises a gene of interest and a selectable marker gene, and

(b)   ligating cleaved expression vectors with non-palindromic ends to produce nucleic acid polymers.

2.   The method of claim 1, wherein the nucleic acid polymers comprise multiple copies of the gene of interest and the selectable marker gene in a 1:1 ratio.

3.   The method of claim 1, further comprising the act of fragmenting the nucleic acid polymer using mechanical shearing.

4.   The method of claim 1, further comprising the act of adding poison oligonucleotides to the cleaved expression vectors having non-palindromic ends before the act of ligating, wherein the poison oligonucleotides are complementary to the non-palindromic ends of the cleaved expression vectors.

5.   The method of claim 1, wherein expression vectors comprising palindromic ends are treated to produce non-palindromic ends by incubating the expression vectors having palindromic ends with an enzyme that provides a 3'-exonuclease activity.

6.   The method of claim 5, wherein the 3'-exonuclease activity-providing enzyme is selected from the group consisting of T4 DNA polymerase, *E. coli* DNA polymerase I, Klenow fragment of DNA polymerase I, DEEP VENT DNA polymerase, and VENT DNA polymerase.

7.   The method of claim 1, wherein the expression vectors comprise a polycistronic transcription unit.

8. The method of claim 1, wherein the selectable marker gene encodes a protein that is titratable.

9. The method of claim 8, wherein the selectable marker gene product is titratable with a toxic molecule.

10. The method of claim 9, wherein the selectable marker gene is selected from the group consisting of a *bleomycin-resistance* gene, a *metallothionein* gene, a *hygromycin B-phosphotransferase* gene, the *AUR1* gene, an *adenosine deaminase* gene, an *aminoglycoside phosphotransferase* gene, a *dihydrofolate reductase* gene, a *thymidine kinase* gene, and a *xanthine-guanine phosphoribosyltransferase* gene.

11. The method of claim 8, wherein the selectable marker gene encodes a protein selected from the group consisting of green fluorescent protein, red fluorescent protein, alkaline phosphatase, CD4, CD8, and Class I major histocompatibility complex protein.

12. The method of claim 1, wherein the expression vectors are cleaved with a class IIS restriction enzyme to provide non-palindromic ends.

13. The method of claim 12, wherein the class IIS restriction enzyme is selected from the group consisting of AccB7I, AceIII, AclWI, AdeI, AhdI, Alw26I, AlwI, AlwNI, ApaBI, AspEI, AspI, AsuHPI, BbsI, BbvI, BbvII, Bce83I, BcefI, BciVI, BfiI, BglI, BinI, BmrI, BpiI, BpmI, BpuAI, BsaI, Bse3DI, Bse4I, BseGI, BseLI, BseRI, BsgI, BslI, BsmAI, BsmBI, BsmFI, BspMI, BsrDI, Bst71I, BstAPI, BstF5I, BstXI, Bsu6I, DraIII, DrdI, DseDI, Eam1104I, Eam1105I, EarI, EchHKI, Eco31I, Eco57I, EcoNI, Esp1396I, Esp3I, FokI, FauI, GsuI, HgaI, HphI, MboII, MsiYI, MwoI, NruGI, PflMI, PflFI, PleI, SfaNI, TspRI, Ksp632I, MmeI, RleAI, SapI, SfiI, TaqII, Tth111I, Tth111II, Van91I, XagI, and XcmI.

14. The method of claim 1, wherein the expression vectors are cleaved to produce non-palindromic ends, using an enzyme selected from the group consisting of AvaI, Ama87I, BcoI, BsoBI, Eco88I, AvaII, Eco47I, Bme18I, HgiEI, SinI, BanI, AccB1I, BshNI, Eco64I, BfmI, BstSFI, SfcI, Bpu10I, BsaMI, BscCI, BsmI, Mva1269I, Bsh1285I, BsaOI, BsiEI, BstMCI, Bse1I, BseNI, BsrI, Cfr10I, BsiI, BssSI, Bst2BI, BsiZI, AspS9I, Cfr13I, Sau96I, Bsp1720I, BlpI, Bpu1102I, CelII, Bst4CI, BstDEI, DdeI, CpoI, CspI, RsrII, DsaI, BstDSI, Eco24I, BanII, EcoT38I, FriOI, HgiJII, Eco130I, StyI, BssT1I, EcoT14I, ErhI, EspI, BlpI,

*Bpu*1102I, *Bsp*1720I, *Cel*II, *Hgi*AI, *Bsi*HKAI, *Alw*21I, *Asp*HI, *Bbv*12I, *Hin*fI, *Psp*PPI, *Ppu*MI, *Psp*5II, *San*DI, *Sdu*I, *Bsp*1286I, *Bmy*I, *Sec*I, *Bsa*JI, *Bse*DI, *Sfc*I, *Bfm*I, *Bst*SFI, and *Sml*I.

      15.   A method for producing a recombinant eukaryotic host cell that expresses a peptide or polypeptide of interest, comprising:

         (a)   cleaving at least two expression vectors to produce either non-palindromic ends or palindromic ends, wherein cleaved expression vectors with palindromic ends are further treated to produce non-palindromic ends, and wherein the expression vector comprises an expression cassette that comprises a gene of interest and a selectable marker gene,

         (b)   ligating cleaved expression vectors with non-palindromic ends to produce nucleic acid polymers,

         (c)   introducing the nucleic acid polymers into a eukaryotic host cell, and

         (d)   culturing the recombinant eukaryotic host cell, which produces the peptide or polypeptide of interest.

      16.   The method of claim 15, wherein the nucleic acid polymers comprise multiple copies of the gene of interest and the selectable marker gene in a 1:1 ratio.

      17.   The method of claim 15, wherein the eukaryotic host cell is selected from the group consisting of a mammalian cell, a fungal cell, an insect cell, and an avian cell.

      18.   A method for producing a recombinant eukaryotic host cell that expresses a peptide or polypeptide of interest, comprising introducing a nucleic acid polymer into a eukaryotic host cell, wherein the nucleic acid polymer comprises multiple expression cassettes with head-to-tail orientations, wherein each expression cassette comprises a gene of interest and a selectable marker gene.

      19.   The method of claim 18, wherein the nucleic acid polymer comprises multiple copies of the gene of interest and a selectable marker gene in an approximate 1:1 ratio.

      20.   The method of claim 18, wherein the selectable marker gene encodes a protein that is titratable.